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# Epstein-Barr virus DNA in serum in a HIV-infected patient during development and treatment of non-Hodgkin's lymphoma

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## Summary

Immunocompromised patients are at risk of developing Epstein-Barr virus (EBV)-related lymphoproliferative disorders. Quantitative determination of serum EBV DNA permits early diagnosis of an AIDS-related non-Hodgkin's lymphoma, and surveillance of treatment response and/or

relapse, and thus may be a useful tool to monitor disease activity.

*Key words:* EBV DNA; HIV; non-Hodgkin's lymphoma

## Introduction

Immunocompromised patients are at risk to develop Epstein-Barr virus (EBV)-associated lymphoproliferative disorders and lymphoma. The incidence of non-Hodgkin's lymphoma (NHL) is increased approximately 100-fold in patients with advanced human immunodeficiency virus (HIV)

infection [1]. Diagnosis is often difficult in early stages, even with modern imaging technology. We report how quantitative assessment of EBV DNA in serum contributed to the diagnosis and follow-up of a NHL in a HIV-infected patient.

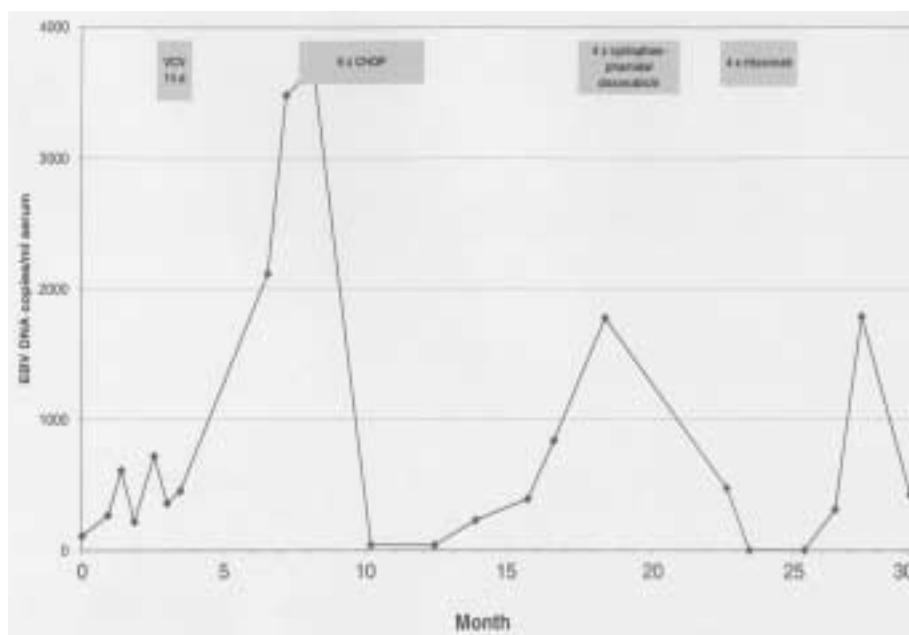
## Case report

A 63-year-old man with haemophilia A had become infected with HIV and hepatitis C, known since 1986 and 1989 respectively. He had a history of diffuse infiltrative lymphocytosis syndrome with bilateral parotid gland swelling (1992) and lymphoid interstitial pneumonitis, which was successfully treated with prednisone (autumn 1999). In January 2001 he presented with fever in the evening, weight loss (5 kg within 3 months), normocytic anaemia (haemoglobin 102 g/L) and rising levels of C-reactive protein (CRP). A chest X-ray was normal, blood and stool cultures remained negative. A computed tomography (CT) revealed multiple retroperitoneal lymph nodes (diameter ~15 mm). His CD4 lymphocyte count was  $186 \times 10^6/L$  (normal:  $>500 \times 10^6/L$ ), the HIV-1 RNA level was 1000 copies/ml. Based on HIV resistance testing, the antiretroviral therapy was changed from zidovudine, didanosine and efavirenz to abacavir, lamivudine and lopinavir/ritonavir. This resulted in sustained full suppression of HIV RNA blood levels and CD4 counts around  $50\text{--}200 \times 10^6/L$ , fever and persisted weight loss. A repeated abdominal CT after three months was unchanged. Serum IgM against EBV early antigen and IgG against EBV nuclear antigen were detected, indicating EBV reactivation. A quantitative real-time polymerase chain reaction (PCR) assay as recently described [2], revealed serum EBV DNA

levels that gradually increased over the next months (figure 1). A 14-day course of oral valacyclovir 1.0 g t.i.d. was unsuccessful, as EBV DNA levels gradually increased up to 3685 copies/ml. The patient deteriorated, developed jaundice and was hospitalised (September 2001). CT now showed progressive enlargement of retroperitoneal lymph nodes of 20 mm in diameter and hepatomegaly without focal lesions. A biopsy of a retroperitoneal lymph node showed immunoblastic lymphocytes positive for CD20 (B cells), the expression of the EBV-encoded latent membrane protein 1 and the expression of Ki-67 >80%, indicating an extensive proliferation rate. The diagnosis of a HIV-associated large cell high grade NHL was made and a cytostatic treatment with cyclophosphamide, doxorubicine, vincristine and prednisone (CHOP regimen) was started (figure 1). Within two weeks the patient's condition improved markedly. Fever, jaundice and inflammatory parameters disappeared. In November, after the third of six chemotherapeutic cycles, the enlarged lymph nodes had completely disappeared on the abdominal CT scan and serum EBV DNA levels fell below the limit of detection (<100 copies/ml). Cytostatic treatment was completed until January 2002. In March, fever in the evening relapsed accompanied by increasing serum EBV DNA levels and normocytic anaemia. CT revealed enlarged

**Figure 1**

Serum levels of EBV DNA measured with a real-time polymerase chain reaction assay in a HIV-positive haemophilic patient. A continuous rise was observed despite an intermittent valacyclovir (VCV) treatment. Nine months later an EBV-associated large B-cell lymphoma was diagnosed, followed by chemotherapy (CHOP), with a complete disappearance of the serum EBV DNA and later re-appearance during relapse. A second chemotherapy (cyclophosphamide and doxorubicin) had no effect, whereas rituximab caused a disappearance again, followed by a new relapse before the patient's death.



retroperitoneal lymph nodes, which prompted a new cytostatic treatment with cyclophosphamide, doxorubicin and prednisone between June and September 2002, without significant clinical improvement. A humanized anti-CD 20 monoclonal antibody (rituximab) treatment (four cycles from December 2002 to March 2003) caused a

marked improvement. Serum EBV DNA levels fell again below the detection limit. In April 2003 serum EBV DNA levels increased again, preceding the clinical relapse with fever, jaundice and progressive deterioration of the general condition. The patient died in July 2003 in hepatic failure. No autopsy was performed.

## Method

Quantitative DNA analysis was performed using the TaqMan® (Applied Biosystems, Rotkreuz, Switzerland) real-time PCR technique. PCR primers and probe were selected for the amplification of a 101 base pair sequence in the conserved BamHI W region of the EBV genome, as reported [2]. Briefly, DNA extracted from 1 ml serum was amplified with 40 cycles of 15 sec. at 95 °C and 60 sec. at 60 °C in an ABI prism 7700 sequence detector using the

TaqMan 2× Universal Master Mix (Applied Biosystems) with 300 nM of each primer and 200 nM of the probe. Each sample was analysed in triplicate, positive and negative controls were included in every run. A pUC18 plasmid containing the EBV BamHI W fragment was included in every PCR run and served as internal control, and was used in the calibration curve based on which the EBV DNA copies were quantified.

## Discussion

EBV-associated lymphoproliferative disorders and lymphoma are a major risk for immunocompromised HIV-infected patients [1]. Highly active antiretroviral therapy (HAART) has contributed to the improved survival rate of patients with HIV infection but has not significantly decreased the incidence of NHL, in contrast to the reduced incidence of Kaposi sarcoma and primary NHL of the central nervous system [3, 4]. The development of quantitative PCR assays permits to monitor the HIV infection and to correlate the serum EBV DNA level with the EBV disease activity, thereby monitoring patients at risk for EBV-associated lymphoproliferation [2, 5–7].

In our patient gradually rising serum EBV DNA levels preceded the clinical detection of the NHL by months. These serum EBV DNA levels correlated well with the EBV disease activity, ie lymphoma. Their time course permitted to mon-

itor and navigate the treatment. Monitoring of EBV DNA levels in blood has been shown to be useful in the diagnosis and management of post-transplant lymphoproliferative disease (PTLD) and other EBV-associated malignancies [8, 9]. However, its predictive value in HIV-infected individuals has been questioned [6, 10]. Most data on EBV burden in HIV patients, treated with HAART or not, are based on EBV levels in peripheral blood mononuclear cells (PBMC) and describe high EBV DNA levels in the majority of HIV infected patients. These high levels result from the expanded pool of EBV-infected cells in these immunocompromised hosts, but do not discriminate patients with AIDS-associated NHL from those without it [6, 10–12]. Our case report suggests that this may be different by following serum levels of EBV DNA, since they heralded the NHL and correlated throughout with the disease

activity. Serum EBV DNA levels indeed have been shown to correlate with EBV disease activity [2, 6, 8, 9] showing a similar sensitivity, but higher specificity than its levels in PBMC from the same patients [8]. Although these findings were not confirmed in other studies, serum EBV DNA levels permitted the detection of NHL patients also in immunocompromised hosts [10, 13].

Some of this controversy may be explained by the different pathogeneses of AIDS-NHL. They include on the one hand Burkitt's lymphoma (BL), which may develop in hosts with a relatively preserved immune function, and which is characterized by consistent c-myc activation and EBV infection in approximately 30%. On the other hand, immunoblastic lymphomas are found only in severely immunodeficient hosts and are associated with EBV in >90%. In contrast to BL, immunoblastic lymphomas express consistently the EBV-encoded latent membrane protein 1, known to function as a viral oncogene. The recent observation that the EBV DNA in the serum of patients with lymphomas and nasopharyngeal carcinomas consists of short DNA fragments [14] suggests that these DNA molecules are released by apoptosis of cancer cells and are not the product of viral replication. This is another argument in favour of quantifying EBV DNA in serum rather than in PBMC.

Pre-emptive treatment of EBV-associated disease in HIV-infected patients according to EBV DNA serum levels has not been examined. As in PTLN, monitoring of EBV DNA may be useful to enable a prompt rather than pre-emptive treatment [15].

The current literature does not identify the best specimen to sample for monitoring of EBV levels in patients at risk. Serum EBV DNA levels are easier to interpret and clinically more useful because EBV DNA is not detected in serum of healthy carriers in contrast to PBMC. Moreover, the levels of serum EBV DNA are independent of the leukocyte count in these frequently cytopenic, immunocompromised patients.

Although large series of patients are needed to confirm the findings of this case, we propose to monitor HIV-infected patients at risk for EBV-associated lymphoproliferation by measuring EBV DNA levels in serum.

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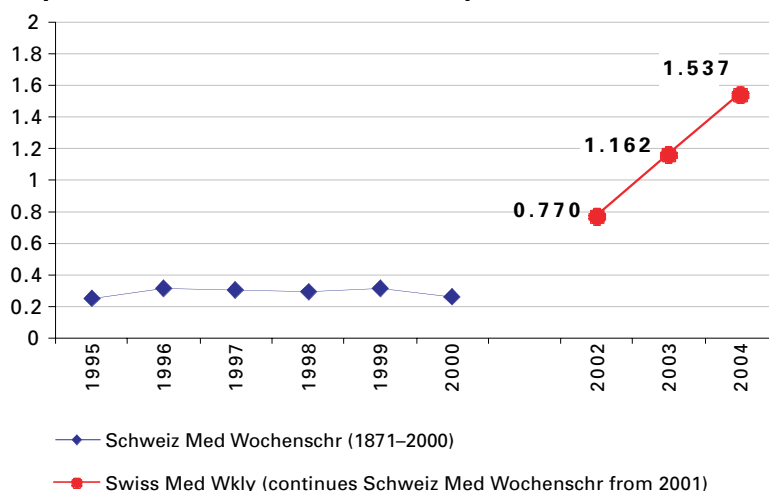
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